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# Model of Chromosomal Instability in Oral Carcinogenesis and Progression

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## 1. Introduction

Epidemiologic and experimental evidence indicate that oral cancer originates and progresses with the contribution of carcinogen exposure, mainly from tobacco smoking (IARC, 1986, 2004). This is thought to contribute to DNA damage within the mucosa and, in particular, to subsequent gene mutations, chromosomal instability and aneuploidy, resulting in an increased risk of developing oral cancer. Accumulation of genetic/genomic aberrations over time leads to a multi-step process of carcinogenesis in which the functions of genes which control the cell cycle (proliferation and apoptosis), chromosome stability, angiogenesis, invasion and metastasis, become aberrant (Califano et al., 1996; Hanahan & Weinberg 2011; Martorell-Calatayud et al., 2009). Chromosomal aberrations in oral cancer are located, in particular, at 9p21, 17p13, 3q26, 11q13, 3p21, 14q32 (Forastiere et al., 2001; Gollin, 2001) corresponding to several putative tumor suppressor genes and oncogenes including p16 at 9p21 and TP53 at 17p13.

Studies aimed at elucidating the steps of transition between the oral precursor lesions and oral cancer and, in particular, the transition from visually normal appearing non-dysplastic oral mucosa to precursor lesions are potentially very informative. Such investigations address the theory of “field cancerization” (Slaughter et al., 1953) and its more recent genetic explanation (Braakhuis et al., 2003; Tabor et al., 2001). These studies have led to a genetic progression model of oral cancer (Braakhuis et al., 2004). A critical step in this model is the conversion of a patch, in which stem cells share genetic/genomic aberrations, into an expanding field in which many more aberrations occur and which sometimes becomes visible as leukoplakias and erythroplakias (Braakhuis et al., 2002; Reid et al., 1997; Van Houten et al., 2000). The role of chromosomal instability during the genesis and progression of oral cancer has clearly been indicated by several studies but still our understanding of the molecular mechanisms is relatively poor. Analyses were performed with the use of different techniques including loss of heterozygosity (Braakhuis et al., 2004; Bremmer et al., 2008; Graveland et al., 2011; Lydiatt et al., 1998; Mithani et al., 2007; Partridge et al., 1997; Tsantoulis et al., 2007), comparative genomic hybridization and gene expression array (Bremmer et al., 2008; Cha et al., 2011; Garnis et al., 2009; Liu et al., 2006, 2011; Smeets et al., 2009; Snijders et al., 2005; Squire et al., 2002;), in situ hybridization (Nees et al., 1993; Voravud et al., 1993), immunohistochemistry (Nees et al., 1993), multiplex ligation-

dependent probe amplification (Bremmer et al., 2008; Cha et al., 2011; Liu et al., 2006), DNA image cytometry (Bremmer et al., 2008; Diwakar et al., 2005) and DNA flow cytometry (DNA FCM) (Donadini et al., 2010; Hemmer, 1990, 1997; Pentenero et al., 2009; Saito, 1998, 1991, 1995; Seoane et al., 1998). DNA FCM was often adopted as a useful technique for detecting the presence of DNA aneuploid sublines in several human predisposing and preneoplastic lesions such as Barrett's esophagus (Reid et al., 2000), ulcerative colitis (Rabinovitch et al., 1999; Risques et al., 2008), colorectal adenomas (Giaretti et al., 1994) and oral lesions (Donadini et al., 2010; Pentenero et al., 2009; Saito, 1998, 1991, 1995; Seoane et al., 1998).

## **2. High resolution DNA FCM of oral lesions and visually normal non-dysplastic mucosa**

The DNA FCM data provided so far for the human oral precancerous lesions were mainly derived from paraffin-embedded material of dysplastic oral potentially malignant lesions (OPMLs). In order to better investigate early oral fields of carcinogenesis and to separate them from later progression steps, we have included the analysis of non-dysplastic "oral clinically normal appearing mucosa sited in OPML and OSCC distant fields within the same subsites" (ODFs;  $n = 122$ ). In addition, we have analyzed multiple samples from OPMLs without and with dysplasia at histology, including also the lesion margins. OPMLs in our series of cases were clinically identified mainly as white lesions of the oral mucosa or leukoplakias ( $n = 235$ ). Further, we analyzed, though in a relatively small number of cases, oral verrucous carcinomas (OVCs;  $n = 9$ ) and oral squamous cell carcinomas (OSCCs;  $n = 32$ ). In all cases the multiple samples were only from fresh/frozen material. Patients were recruited in three different medical centers: the Oral Medicine and Oral Oncology Section of the University of Turin, the Department of Otolaryngology, "S. Martino Hospital" in Genoa and the National Institute for Cancer Research in Genoa. Patient written consent was obtained in every case according to the Institutional Ethic Committees. Diagnosis in every case was obtained from the Pathology Departments of the same Institutions. In particular, the diagnosis of OPMLs, using both incisional biopsies and/or microbiopsies as previously detailed (Navone et al., 2008) was based on internationally accepted criteria with levels of diagnostic certainty C3-C4 (Van der Waal et al., 2009). The assessment of the degree of dysplasia was carried out by a specially trained pathologist according to the WHO guidelines (IARC, 2005). Tissue fragments were minced on Petri dishes using scalpels and collected in 2 ml detergent solution (0.1 M citric acid, 0.5% Tween-20) (Otto, 1994) and then submitted to mechanical disaggregation in a disposable 50  $\mu\text{m}$  Medicon using a Medimachine (DAKO, Copenhagen, Denmark). Nuclei suspensions were obtained and filtered over a 50  $\mu\text{m}$  nylon sieve (CellTrics, Partec GmbH, Muenster, Germany). An absolute count of the nuclei in suspension was performed by FCM (CyFlow® ML, Partec GmbH (Shapiro, 2003)) after 1 to 10 dilution in water. The final volume was calculated to obtain the concentration of 600,000 nuclei/ml. One volume (1/7 of the final volume) of detergent solution was first added followed by 10 min incubation and gentle shaking. Finally, 6 volumes (6/7 of the final volume) of staining solution (0.4 M  $\text{Na}_2\text{HPO}_4$ , 5  $\mu\text{M}$  DAPI in water) were added. Samples were kept on dark for a minimum of 15 min incubation before filtering and FCM analysis. Excitation of DAPI was provided with an UV mercury lamp (HBO-100 W, Partec GmbH) and the emitted blue fluorescence was collected

using a 435 nm long-pass filter. Measurements of DNA content histograms were performed with a high resolution DNA FCM (CyFlow® ML, PartecGmbH (Shapiro, 2003)) according to quality controls and analysis consensus criteria (Ormerod et al., 1998). Only samples with at least 2 separate G0-G1 peaks were considered DNA aneuploid. Sex specific human lymphocytes and “true oral normal mucosa” from healthy donors were used as DNA diploid controls. DNA Index (DI) values were evaluated as the ratio of the mean channel number of the DNA aneuploid G0-G1 peak to the mean channel number of the DNA diploid G0-G1 peak. Thus, DNA diploid and aneuploid sublines have values respectively  $DI = 1$  and  $DI \neq 1$ . The CV values of the G0-G1 peaks for the DNA diploid normal mucosa samples from healthy donors were used as a measure of accuracy (DNA resolution): a mean  $CV = 1.88 \pm 0.26\%$  was obtained by a Gaussian curve fitting method (FloMax Software 3.0b4 2001, Partec GmbH). The mean CV value using human lymphocytes from sex specific healthy donors was  $1.2 \pm 0.2\%$ . Data collection, management and analyses were done using Microsoft Office Excel and the SPSS 16.0 software package (Apache Software Foundation, Chicago, IL, USA). The association between two variables in  $2 \times 2$  contingency tables was evaluated with the Fisher exact test. A p-value  $< 0.05$  was taken as statistically significant.

Table 1a shows the prevalence of DNA aneuploidy for 7 subgroups of oral mucosa/lesions: “true normal mucosa” from healthy donors ( $n = 36$ ), non-dysplastic ODFs corresponding to OPMLs ( $n = 105$ ), and OPMLs without ( $n = 208$ ) and with dysplasia ( $n=27$ ) were DNA aneuploid respectively in none of the cases, in 12/105 (11.4%), 37/208 (17.8%) and in 10/27 (37%). The samples relative to advanced cancer, respectively OVCs and OSCCs, were DNA aneuploid in 6/9 (66.7%) and 25/32 (78.1%) cases. ODFs corresponding to OSCCs were DNA aneuploid in 3/17 (17.6%) cases. Two or more DNA aneuploid sublines were detected in none of the ODFs, in 5/37 (13.5%) of the OPMLs without dysplasia, in 2/10 (20%) of the OPMLs with dysplasia. OVCs and OSCCs presented respectively multiple DNA aneuploid sublines in 2/6 (33.3%) and in 13/25 (52%) cases.

Oral mucosa/lesion groups	N. cases within groups	N. DNA aneuploid cases	One DNA aneuploid subline	Two or more DNA aneuploid sublines
"True normal mucosa" from healthy donors	36	0 (0%)	-	-
ODFs corresponding to OPMLs	105	12 (11.4%)	12 (100%)	0 (0%)
OPMLs without dysplasia	208	37 (17.8%)	32 (86.5%)	5 (13.5%)
OPMLs with dysplasia	27	10 (37.0%)	8 (80.0%)	2 (20.0%)
OVCs	9	6 (66.7%)	4 (66.7%)	2 (33.3%)
ODFs corresponding to OSCCs	17	3 (17.6%)	3 (100%)	0 (0%)
OSCCs	32	25 (78.1%)	12 (48.0%)	13 (52.0%)

Abbreviations: ODFs = non-dysplastic oral clinically normal appearing mucosa sited in OPML and OSCC distant fields within the same anatomical subsites; OPMLs = Oral Potentially Malignant Lesions (mainly leukoplakias); OVCs = Oral Verrucous Carcinomas; OSCCs = Oral Squamous Cell Carcinomas.

Table 1a. DNA aneuploidy by high resolution DNA FCM among 7 different groups of oral lesions and non-dysplastic normal appearing mucosa. The last 2 columns report the cases with single and multiple DNA aneuploid sublines.

All the DI aneuploid sublines ( $n = 126$ ) were subdivided in 2 classes: DNA near-diploid ( $DI \neq 1$  and  $<1.4$ ) and DNA high aneuploid ( $DI \geq 1.4$ ) (Table 1b). ODFs and OPMLs without dysplasia were characterized by near-diploid sublines respectively in 12/12 (100%) and in 38/43 (88.4%) of the cases. OVCs had a significantly higher frequency of DNA near-diploid aneuploid cases than OSCCs (respectively, 87.5% and 33.3%;  $p=0.006$ ). In contrast, OPMLs with dysplasia and OSCCs had high aneuploid sublines respectively in 5 out of 12 (41.7%) and in 32 out of 48 (66.7%) of the cases. The prevalence of high aneuploidy in OPMLs with dysplasia was statistically significantly higher than in OPMLs without dysplasia ( $p = 0.03$ ).

Oral mucosa/lesion groups	N. DNA aneuploid sublines	N. DNA near-diploid aneuploid sublines (DI≠1 and DI<1.4)	N. DNA high aneuploid sublines (DI≥1.4)
"True normal mucosa" from healthy donors	-	-	-
ODFs corresponding to OPMLs	12	12 (100%)	0 (0%)
OPMLs without dysplasia	43	38 (88.4%)	5 (11.6%)
OPMLs with dysplasia	12	7 (58.3%)	5 (41.7%)
OVCs	8	7 (87.5%)	1 (12.5%)
ODFs corresponding to OSCCs	3	2 (66.7%)	1 (33.3%)
OSCCs	48	16 (33.3%)	32 (66.7%)

Abbreviations: ODFs = non-dysplastic oral clinically normal appearing mucosa sited in OPML and OSCC distant fields within the same anatomical subsites; OPMLs = Oral Potentially Malignant Lesions (mainly leukoplakias); OVCs = Oral Verrucous Carcinomas; OSCCs = Oral Squamous Cell Carcinomas.

Table 1b. Presence of DNA near-diploid ( $DI \neq 1$  and  $DI < 1.4$ ) and high aneuploid ( $DI \geq 1.4$ ) sublines among 7 different groups of oral mucosa/lesions. DNA aneuploidy was measured by high resolution DNA FCM.

3. Discussion

The incidence of DNA aneuploidy by FCM reported in the literature ranges from about 10% to 40% for dysplastic OPMLs (Donadini et al. 2010; Pentenero et al. 2009; Saito et al., 1998, 1991, 1995; Seoane et al., 1998) and up to about 80% for OSCCs (Donadini et al., 2010; Pentenero et al. 2009; Hemmer, 1990, 1997). These values may strongly depend on the tissue material type (paraffin embedded or fresh-frozen) and DNA FCM resolution. In the present study, partly based on a previous data set that was already published (Donadini et al., 2010), we have performed FCM measurements at optimized conditions (fresh-frozen material, concentration of 600,000 nuclei/ml, DAPI staining in nuclei suspensions, UV incident light, the use of a dedicated instrument). Correspondingly, the CV values of the G0-G1 peaks of human normal control DNA diploid nuclei were commonly near 1%, while a minimum DNA change of 2.4% was detected (Figure 1). It is likely that DNA FCM at lower resolution and higher CV values would not allow separating DNA near-diploid aneuploid sublines with only slight DNA changes above/below DNA diploidy.

The present data set confirmed in a larger number of cases that, while “true normal oral mucosa” and human lymphocytes of healthy donors were DNA diploid in all cases, non-dysplastic “clinically normal appearing mucosa fields of the oral cavity” (ODFs) in patients with OPMLs already contained DNA aneuploid sublines in a subgroup of cases (12/105, 11%).



Moreover, it was found that OPMLs that could be clinically identified mainly as white lesions of the oral mucosa (leukoplakias) and classified without dysplasia at histology, contained already DNA aneuploid sublines in 37/208 (18%) of the cases. These data appear in agreement with the concept of field effect in oral carcinogenesis (Braakhuis et al., 2003; Bremmer et al., 2008; Leemans, 2011; Tabor et al., 2001; Van der Waal, 1997). The data obtained for the non-dysplastic OPMLs, in particular, were in agreement with previous literature reports including two studies from our group using an independent patient population (Donadini et al., 2010; Pentenero et al., 2009; Saito, 1995). These data were, however, in contrast with other studies, which did not detect DNA aneuploid sublines in such lesions (Kahn et al., 1992; Saito, 1998). The present study has additionally highlighted that ODFs and OPMLs without dysplasia were characterized by single near-diploid DNA aneuploid sublines. On the contrary, OPMLs with dysplasia contained high DNA aneuploid sublines ( $DI \geq 1.4$ ) in slightly less than half of the cases (42%). High DNA aneuploid sublines were predominant (67%) for the OSCCs, which were in addition characterized by the presence of multiple DNA aneuploid sublines in 52% of the DNA aneuploid cases. In contrast, OVCs were characterized by DNA near-diploid aneuploid sublines in 67% of the cases in agreement with previously published data (Pentenero et al., 2011). Overall, the present data support a previous model of aneuploidy genesis and evolution (Giaretti, 1994). Accordingly, a transition from DNA diploidy to near-diploid aneuploidy would be an early step of the natural history of OPMLs, while high DNA aneuploidy (likely to derive from the endoreduplication of a DNA hypo-diploid or hyper-diploid near-diploid cell) would frequently occur as a later event in OPMLs with dysplasia and OSCCs (Figure 1). From the clinical point of view, one can speculate that the detection of DNA content genomic aberrations in oral fields, which appear visually and histologically normal, and in OPMLs may have profound implications for improvement of the present patient management by identifying individuals at high risk to develop cancer (Brennan et al., 2007; Dakubo et al., 2007; Lodi et al., 2006).

Moreover, it is possible, though still unproved, that the OSCC group in which near-diploid aneuploid DIs remained “frozen” during time are at better prognosis compared to OSCCs with multiple DNA aneuploid sublines with high DNA aneuploidy. Interestingly, OVCs that were mainly characterized by DI values in the near-diploid region are known to be less aggressive and at better prognosis than OSCCs.

Clearly, what is still strongly needed in the model system of oral preneoplasia and neoplasia is a better understanding of the origin and dynamic evolution of chromosomal instability, chromosomal aberrations and aneuploidy (Albertson et al., 2003; Asteriti et al., 2010; Compton et al., 2011; Geigl et al., 2008; Giet et al., 2005; Kops et al., 2005; Lingen et al., 2011; Sieber et al., 2003; Suijkerbuijk & Kops, 2008; Thompson et al., 2010; Viet & Schmidt, 2010). In other models of cancer genesis and progression, like the colorectal adenoma-carcinoma sequence, the Barrett's esophagus and the ulcerative colitis transition to carcinoma, the role of APC and TP53 has been highlighted (Fodde et al., 2001; Giaretti et al., 2004; Rabinovitch et al., 2004). A role of TP53 in oral cancer chromosomal instability (Negrini et al., 2010) is also likely to occur due to different sources of TP53 inactivation including HPV infection in different sites of the oral cavity (Leemans, 2011; Klingelhutz et al., 2005; Tsantoulis et al., 2007). Studies that linked the genome-wide integrity analysis with gene expression profiles have provided powerful indications that chromosomal instability and aneuploidy massively deregulate the cellular transcriptome (Albertson et al., 2003). Future studies coupling both

these techniques are likely to contribute to discover specific recurrent genomic aberrations, which encompass specific genes with a potential role in the genesis of chromosomal instability and aneuploidy. The functional consequences of specific DNA gains/losses are, however, not only involving oncogenes and tumor suppressor genes. More subtle and complex mechanisms are present since many aberrations span large chromosomal regions including normal genes, which coordinately and cooperatively may influence important cell functions as proliferation, differentiation, apoptosis and DNA repair.

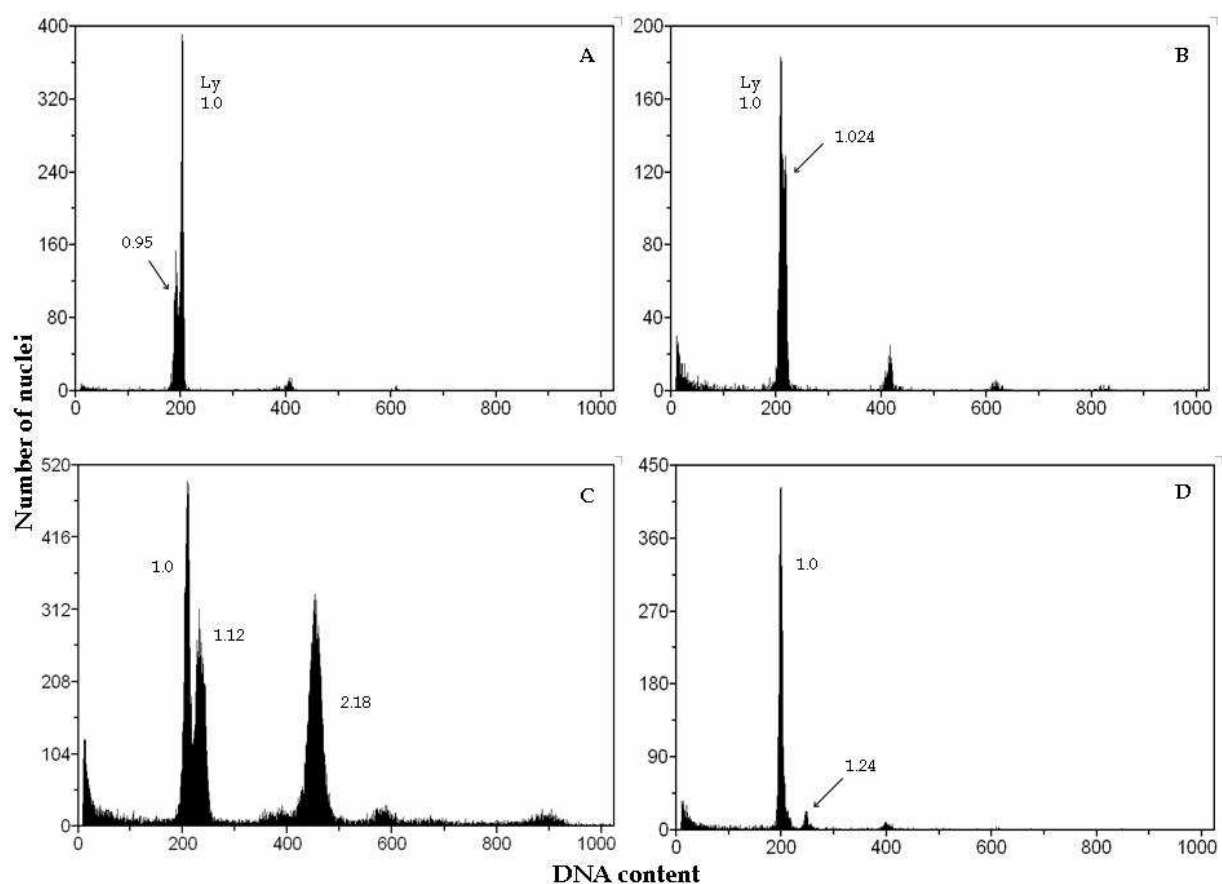


Fig. 1. Examples of DNA content histograms from oral fresh/frozen mucosa/lesions as obtained by low background and high resolution DNA FCM. A model of DNA aneuploidization and evolution.

Single DNA aneuploid sublines in the DNA near-diploid aneuploid region ( $DI \neq 1$  and  $DI < 1.4$ ) are shown in A and B for two OPMLs. Sex specific human lymphocytes (Ly) and true normal mucosa of healthy donors were used as DNA diploid controls ( $DI = 1.0$ ). Multiple DNA aneuploid sublines for an OSCC with DI values respectively of 1.12 and 2.18 are shown in C. This example illustrates a model of DNA aneuploidisation as previously reported (Giaretti, 1994; Donadini et al., 2010). The key mechanism of DNA aneuploidisation appears related to a loss of symmetry of DNA content during an abnormal mitotic division in which the two daughter cells loose or gain respectively a small amount of DNA and generate DNA near-diploid aneuploid sublines (see examples A and B). A second

step of DNA aneuploidy evolution appears to be due to the endoreduplication of the DNA near-diploid aneuploid cells sublines (see example C). The large G0-G1 subpopulation of cells with  $DI = 2.18$  is characterized by a relatively large CV value (about 3% with respect to 2% of the G0-G1 DNA diploid peak), which is indicative of chromosomal instability and potential loss of DNA. The  $DI = 2.18$  value of the high DNA aneuploid G0-G1 peak was likely originated from an initial DI value of 2.24 (twice as much of the original near-diploid  $DI = 1.12$ ) with the loss of 2.7% DNA.

An example of DNA histogram with a small G0-G1 DNA aneuploid peak (with 5% of the total number of nuclei in this case) and with  $DI = 1.24$  is shown in D to illustrate that such a small percentage of DNA aneuploid nuclei can be sufficient to be detected by our high resolution and low background DNA FCM measurements. This approach was characterized by the use of fresh/frozen tissue material, nuclei suspensions at the fixed concentration of 600,000 nuclei/ml, DAPI staining and a dedicated FCM instrument (see details in the text; Donadini et al., 2010; Shapiro, 2003).

#### 4. Acknowledgements

This study has been supported by “Compagnia di San Paolo - Programma Oncologia” and by Philip Morris International. Thanks are due, in particular, to A. Donadini, M. Maffei and P. Castagnola for critically reading the manuscript.

#### 5. Refereneces

- Albertson, D.G., Collins, C., McCormick, F. & Gray, J.W. (2003). Chromosome aberrations in solid tumors. *Nat. Genet.* 34, pp 369-376.
- Asteriti, I.A., Rensen, W.M., Lindon, C., Lavia, P. & Guarguaglini, G. (2010). The Aurora-A/TPX2 complex: a novel oncogenic holoenzyme? *Biochim. Biophys. Acta.* 1806, pp 230-239.
- Braakhuis, B.J., Tabor, M.P., Leemans, C.R., Van der Waal, I., Snow, G.B. & Brakenhoff, R.H. (2002). Second primary tumors and field cancerization in oral and oropharyngeal cancer: molecular techniques provide new insights and definitions. *Head Neck.* 24, pp 198-206.
- Braakhuis, B.J., Tabor, M.P., Kummer, J.A., Leemans, C.R. & Brakenhoff, R.H. (2003). A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res.* 63, pp 1727-1730.
- Braakhuis, B.J., Leemans, C.R. & Brakenhoff, R.H. (2004). A genetic progression model of oral cancer: current evidence and clinical implications. *J. Oral Pathol. Med.* 33, pp 317-322.
- Bremmer, J.F., Braakhuis, B.J., Brink, A., Broeckaert, M.A., Belien, J.A., Meijer, G.A., Kuik, D.J., Leemans, C.R., Bloemena, E., Van der Waal, I. & Brakenhoff, R.H. (2008). Comparative evaluation of genetic assays to identify oral pre-cancerous fields. *J. Oral Pathol. Med.* 37, pp 599-606.
- Brennan, M., Migliorati, C.A., Lockhart, P.B., Wray, D., Al-Hashimi, I., Axell, T., Bruce, A.J., Carpenter, W., Eisenberg, E., Epstein, J.B., Holmstrup, P., Jontell, M., Nair, R., Sasser, H., Schifter, M., Silverman, B., Thongprasom, K., Thornhill, M., Warnakulasuriya, S. & Van der Waal, I. (2007). Management of oral epithelial



- dysplasia: a review. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 103 Suppl, S19 e11-12
- Califano, J., van der Riet, P., Westra, W., Nawroz, H., Clayman, G., Piantadosi, S., Corio, R., Lee, D., Greenberg, B., Koch, W. & Sidransky, D. (1996). Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res.* 56, pp 2488-2492.
- Cha, J.D., Kim, H.J. & Cha, I.H. (2011). Genetic alterations in oral squamous cell carcinoma progression detected by combining array-based comparative genomic hybridization and multiplex ligation-dependent probe amplification. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 111(5), (2011 May), pp 594-607.
- Compton D.A. (2011). Mechanisms of aneuploidy. *Curr Opin Cell Biol.* 23(1), (2011 Feb), pp 109-13.
- Dakubo, G.D., Jakupciak, J.P., Birch-Machin, M.A. & Parr, R.L. (2007). Clinical implications and utility of field cancerization. *Cancer Cell. Int.* 7, 2.
- Donadini, A., Maffei, M., Cavallero, A., Pentenero, M., Malacarne, D., Di Nallo, E., Truini, M., Navone, R., Mereu, P., Scala, M., Santelli, A., Gandolfo, S., & Giaretti, W. (2010). Oral cancer genesis and progression: DNA near-diploid aneuploidization and endoreduplication by high resolution flow cytometry. *Cell. Oncol.* 32, pp 373-383.
- Fodde, R., Kuipers, J., Rosenberg, C., Smits, R., Kielman, M., Gaspar, C., van Es, J.H., Breukel, C., Wiegant, J., Giles, R.H. & Clevers, H. (2001). Mutations in the APC tumour suppressor gene cause chromosomal instability. *Nat. Cell Biol.* 3, pp 433-438.
- Forastiere, A., Koch, W., Trotti, A. & Sidransky, D. (2001). Head and neck cancer. *N. Engl. J. Med.* 345, pp 1890-1900.
- Geigl, J.B., Obenaus, A.C., Schwarzbach, T. & Speicher, M.R. (2008). Defining 'chromosomal instability'. *Trends Genet.* 24(2), (2008 Feb), pp 64-9.
- Giaretti, W. (1994). A model of DNA aneuploidization and evolution in colorectal cancer. *Lab. Invest.* 71, pp 904-910.
- Giaretti, W., Venesio, T., Prevosto, C., Lombardo, F., Ceccarelli, J., Molinu, S. & Risio, M. (2004). Chromosomal instability and APC gene mutations in human sporadic colorectal adenomas. *J. Pathol.* 204, pp 193-199.
- Giet, R., Petretti, C. & Prigent, C. (2005). Aurora kinases, aneuploidy and cancer, a coincidence or a real link? *Trends Cell Biol.* 15, pp 241-250.
- Graveland, A.P., Golusinski, P.J., Buijze, M., Douma, R., Sons, N., Kuik, D.J., Bloemena, E., Leemans, C.R., Brakenhoff, R.H. & Braakhuis, B.J. (2011). Loss of heterozygosity at 9p and p53 immunopositivity in surgical margins predict local relapse in head and neck squamous cell carcinoma. *Int J Cancer.* 128(8), (2011 Apr), pp 1852-9.
- Gollin, S.M. (2001) Chromosomal alterations in squamous cell carcinomas of the head and neck: window to the biology of disease. *Head Neck.* 23, pp 238-253.
- Hanahan, D. & Weinberg, R.A. (2011). Hallmarks of Cancer: The Next Generation. *Cell.* 144, pp 646-74
- Hemmer, J. & Kreidler, J. (1990). Flow cytometric DNA ploidy analysis of squamous cell carcinoma of the oral cavity. Comparison with clinical staging and histologic grading. *Cancer.* 66, pp 317-320.

- Hemmer, J., Thein, T. & Van Heerden, W.F. (1997). The value of DNA flow cytometry in predicting the development of lymph node metastasis and survival in patients with locally recurrent oral squamous cell carcinoma. *Cancer*. 79, pp 2309-2313.
- International Agency for Research on Cancer (1986). *Tobacco smoking*. IARC Monogr Eval Carcinog Risk Chem Hum. 38, pp 35-394.
- International Agency for Research on Cancer (2004). *Tobacco smoke and involuntary smoking*, IARC Monogr Eval Carcinog Risk Chem Hum. pp 1-1438.
- International Agency for Research on Cancer (2005). *Pathology and Genetics of Head and Neck Tumours..* IARC Monogr Eval Carcinog Risk Chem Hum.
- Kahn, M.A., Dockter, M.E. & Hermann-Petrin, J.M. (1992). Flow cytometer analysis of oral premalignant lesions: a pilot study and review. *J. Oral Pathol. Med.* 21, pp 1-6.
- Klingelhutz, A.J., Qian, Q., Phillips, S.L., Gourronc, F.A., Darbro, B.W. & Patil, S.R. (2005). Amplification of the chromosome 20q region is associated with expression of HPV-16 E7 in human airway and anogenital epithelial cells. *Virology*. 30,340(2), (2005 Sep), pp 237-44.
- Kops, G.J., Weaver, B.A. & Cleveland, D.W. (2005). On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat. Rev. Cancer*. 5, pp 773-785.
- Leemans, C.R., Braakhuis, B.J., Brakenhoff, R.H. (2011). The molecular biology of head and neck cancer. *Nat Rev Cancer*. 11(1), (Jan 2011), pp 9-22.
- Lingen, M.W., Pinto, A., Mendes, R.A., Franchini, R., Czerninski, R., Tilakaratne, W.M., Partridge, M., Peterson, D.E. & Woo, S.B. (2011). Genetics/epigenetics of oral premalignancy: current status and future research. *Oral Dis*. 17 Suppl 1, (2011 Apr), pp 7-22.
- Liu, C.J., Lin, S.C., Chen, Y.J., Chang, K.M. & Chang, K.W.(2006). Array-comparative genomic hybridization to detect genome wide changes in microdissected primary and metastatic oral squamous cell carcinomas. *Mol Carcinog*. 45(10), (2006 Oct), pp 721-31.
- Liu, W., Zheng, W., Xie, J., Zhang, B., Ma, W. & Chen, X. (2011). Identification of genes related to carcinogenesis of oral leukoplakia by oligo cancer microarray analysis. *Oncol Rep.*;26(1), (2011 Jul), pp 265-74.
- Lodi, G., Sardella, A., Bez, C., Demarosi, F. & Carrassi, A. (2006). Interventions for treating oral leukoplakia. *Cochrane Database Syst. Rev*. CD001829.
- Lydiatt, W.M., Anderson, P.E., Bazzana, T., Casale, M., Hughes, C.J., Huvos, A.G., Lydiatt, D.D. & Schantz, S.P. (1998). Molecular support for field cancerization in the head and neck. *Cancer*. 82, pp 1376-1380.
- Martorell-Calatayud, A., Botella-Estrada, R., Bagan-Sebastian, O., Sanmartin-Jimenez, J.V. & Guillen-Barona, C. (2009). Oral leukoplakia: clinical, histopathologic, and molecular features and therapeutic approach. *Actas Dermosifiliogr*. 100, pp 669-684.
- Mithani, S.K., Mydlarz, W.K., Grumbine, F.L., Smith, I.M. & Califano, J.A. (2007). Molecular genetics of premalignant oral lesions. *Oral Dis*. 13(2), (2007 Mar), pp 126-33.
- Navone, R., Pentenero, M., Rostan, I., Burlo, P., Marsico, A., Broccoletti, R., Scully, C. & Gandolfo, S. (2008). Oral potentially malignant lesions: first-level micro-histological diagnosis from tissue fragments sampled in liquid-based diagnostic cytology. *J. Oral Pathol. Med*. 37, pp 358-363.
- Nees, M., Homann, N., Discher, H., Andl, T., Enders, C., Herold-Mende, C., Schuhmann, A. & Bosch, F.X. (1993). Expression of mutated p53 occurs in tumor-distant epithelia of

- head and neck cancer patients: a possible molecular basis for the development of multiple tumors. *Cancer Res.* 53, pp 4189-4196.
- Negrini, S., Gorgoulis, V.G. & Halazonetis, T.D. (2010). Genomic instability: an evolving hallmark of cancer. *Nat Rev Mol Cell Biol.* 11(3), (2010 Mar), pp 220-8.
- Ormerod, M.G., Tribukait, B., & Giaretti, W. (1998). Consensus report of the task force on standardisation of DNA flow cytometry in clinical pathology. DNA Flow Cytometry Task Force of the European Society for Analytical Cellular Pathology, *Anal. Cell. Pathol.* 17, pp 103-110.
- Otto, F.J. (1994). High-resolution analysis of nuclear DNA employing the fluorochrome DAPI, in *Methods Cell Biol.*, ASCB, pp. 211-217, Academic Press, San Diego.
- Partridge, M., Emilion, G., Pateromichelakis, S., Phillips, E. & Langdon, J. (1997). Field cancerisation of the oral cavity: comparison of the spectrum of molecular alterations in cases presenting with both dysplastic and malignant lesions. *Oral Oncol.* 33(5), (1997 Sep), pp 332-7.
- Pentenero, M., Giaretti, W., Navone, R., Demurtas, A., Rostan, I., Bertolusso, G., Broccoletti, R., Arduino, P.G, Malacarne, D. & Gandolfo, S. (2009). DNA aneuploidy and dysplasia in oral potentially malignant disorders: association with cigarette smoking and site. *Oral Oncol.* 45, pp 887-890.
- Pentenero, M., Donadini, A., Di Nallo, E., Maffei, M., Marino, R., Familiari, U., Broccoletti, R., Castagnola, P., Gandolfo, S. & Giaretti, W. (2011). Distinctive chromosomal instability patterns in oral verrucous and squamous cell carcinomas detected by high-resolution DNA flow cytometry. *Cancer.* 117(22), pp 5052-7.
- Rabinovitch, P.S., Dziadon, S., Brentnall, T.A., Emond, M.J., Crispin, D.A., Haggitt R.C. & Bronner, N.P. (1999). Pancolonic chromosomal instability precedes dysplasia and cancer in ulcerative colitis. *Cancer Res.* 59, pp 5148-5153.
- Rabinovitch, P.S. (2004). Genomic instability and progression to cancer in two models of preneoplastic disease: Barrett's esophagus and ulcerative colitis. *Cell. Oncol.* 26, pp 210-211.
- Reid, B.J., Levine, D.S., Longton, G., Blount, P.L. & Rabinovitch, P.S. (2000). Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets, *Am. J. Gastroenterol.* 95, pp 1669-1676.
- Reid, C.B., Snow, G.B., Brakenhoff, R.H. & Braakhuis, B.J. (1997). Biologic implications of genetic changes in head and neck squamous cell carcinogenesis. *Aust. N Z J Surg.* 67, pp 410-416.
- Risques, R.A., Lai, L.A., Brentnall, T.A., Li, L., Feng, Z., Gallaher, J., Mandelson, M.T., Potter, J.D., Bronner, M.P. & Rabinovitch, P.S. (2008). Ulcerative colitis is a disease of accelerated colon aging: evidence from telomere attrition and DNA damage. *Gastroenterology* 135, pp 410-418.
- Saito, T., Notani, K., Miura, H., Fukuda, H., Mizuno, S., Shindoh, M. & Amemiya, A. (1991). DNA analysis of oral leukoplakia by flow cytometry. *Int. J. Oral Maxillofac. Surg.* 20, pp 259-263.
- Saito, T., Yamashita, T., Notani, K., Fukuda, H., Mizuno, S., Shindoh, M. & Amemiya, A. (1995). Flow cytometric analysis of nuclear DNA content in oral leukoplakia: relation to clinicopathologic findings. *Int. J. Oral Maxillofac. Surg.* 24, pp 44-47.

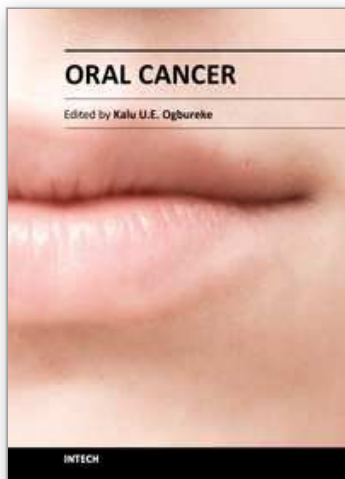
- Saito, T., Mizuno, S., Notani, K., Fukuda, H., Kobayashi, I., Shindoh, M. & Kohgo, T. (1998). Flow cytometric analysis of cell cycle fractions in oral leukoplakia. *Int. J. Oral Maxillofac. Surg.* 27, pp 217-221.
- Seoane, J., Bascones, A., Asenjo, J.A., Garcia-Pola, M. & Varela-Centelles, P.I. (1998). Flow cytometric analysis of nuclear DNA content in oral leukoplakia. *Clin. Otolaryngol. Allied. Sci.* 23, pp 136-140.
- Shapiro, H.M. (2003). Partec GmbH, in: *Practical Flow Cytometry*, 4th edn, pp. 427-428, Wiley, Hoboken, New Jersey.
- Sieber, O.M., Heinimann, K. & Tomlinson, I.P. (2003). Genomic instability : the engine of tumorigenesis? *Nat Rev Cancer.* 3(9), (2003 Sep), pp 701-8.
- Slaughter, D.P., Southwick, H.W., Smejkal, W. (1953). Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. *Cancer* 6, pp 963-968.
- Smeets, S.J., Brakenhoff, R.H., Ylstra, B., Van Wieringen, W.N., Van de Wiel, M.A., Leemans, C.R. & Braakhuis, B.J. (2009). Genetic classification of oral and oropharyngeal carcinomas identifies subgroups with a different prognosis. *Cell Oncol.* 31(4), pp 291-300.
- Snijders, A.M., Schmidt, B.L., Fridlyand, J., Dekker, N., Pinkel, D., Jordan, R.C. & Albertson, D.G. (2005). Rare amplicons implicate frequent deregulation of cell fate specification pathways in oral squamous cell carcinoma. *Oncogene.* 16, 24(26), pp 4232-42.
- Squire, J.A., Bayani, J., Luk, C., Unwin, L., Tokunaga, J., MacMillan, C., Irish, J., Brown, D., Gullane, P. & Kamel-Reid, S. (2002). Molecular cytogenetic analysis of head and neck squamous cell carcinoma: By comparative genomic hybridization, spectral karyotyping, and expression array analysis. *Head Neck.* 24(9), (2002 Sep ), pp 874-87.
- Suijkerbuijk, S.J. & Kops, G.J.(2008). Preventing aneuploidy: the contribution of mitotic checkpoint proteins. *Biochim Biophys Acta.* 1786(1), (2008 Sep), pp 24-31.
- Tabor, M.P., Brakenhoff, R.H., van Houten, V.M., Kummer, J.A., Snel, M.H., Snijders, P.J., Snow, G.B., Leemans, C.R. & Braakhuis, B.J. (2001). Persistence of genetically altered fields in head and neck cancer patients: biological and clinical implications. *Clin. Cancer Res.* 7, pp 1523-1532.
- Thompson, S.L., Bakhoum, S.F. & Compton, D.A. (2010). Mechanisms of chromosomal instability. *Curr Biol.* 23, 20(6), (2010 Mar), pp R285-95.
- Tsantoulis, P.K., Kastrinakis, N.G., Tourvas, A.D., Laskaris, G. & Gorgoulis, V.G. (2007). Advances in the biology of oral cancer. *Oral Oncol.* 43(6), (2007 Jul), pp 523-34.
- Van der Waal, I., Schepman, K.P., Van der Meij, E.H. & Smeele, L.E. (1997). Oral leukoplakia: a clinicopathological review. *Oral Oncol.* 33, pp 291-301.
- Van der Waal, I. (2009). Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. *Oral Oncol.* 45, pp 317-323.
- Van Houten, V.M., Tabor, M.P., Van den Brekel, M.W., Denkers, F., Wishaupt, R.G., Kummer, J.A., Snow, G.B. & Brakenhoff, R.H. (2000). Molecular assays for the diagnosis of minimal residual head-and-neck cancer: methods, reliability, pitfalls, and solutions. *Clin. Cancer Res.* 6, pp 3803-3816.
- Viet, C.T. & Schmidt B.L. (2010). Understanding oral cancer in the genome era. *Head Neck.* 32(9), (2010 Sep), pp 1246-68.

Voravud, N., Shin, D.M., Ro, J.Y., Lee, J.S., Hong, W.K. & Hittelman, W.N. (1993). Increased polysomies of chromosomes 7 and 17 during head and neck multistage tumorigenesis. *Cancer Res.* 53, pp 2874-2883.

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## **Oral Cancer**

Edited by Dr. Kalu U. E. Ogbureke

ISBN 978-953-51-0228-1

Hard cover, 388 pages

**Publisher** InTech

**Published online** 14, March, 2012

**Published in print edition** March, 2012

Oral cancer is a significant public health challenge globally. Although the oral cavity is easily accessible, early diagnosis remains slow compared to the enhanced detection of cancers of the breast, colon, prostate, and melanoma. As a result, the mortality rate from oral cancer for the past four decades has remained high at over 50% in spite of advances in treatment modalities. This contrasts with considerable decrease in mortality rates for cancers of the breast, colon, prostate, and melanoma during the same period. This book attempts to provide a reference-friendly update on the etiologic/risk factors, current clinical diagnostic tools, management philosophies, molecular biomarkers, and progression indicators of oral cancer.

### **How to reference**

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Walter Giaretti (2012). Model of Chromosomal Instability in Oral Carcinogenesis and Progression, Oral Cancer, Dr. Kalu U. E. Ogbureke (Ed.), ISBN: 978-953-51-0228-1, InTech, Available from:  
<http://www.intechopen.com/books/oral-cancer/model-of-chromosomal-instability-in-oral-carcinogenesis-and-progression>

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